

Application No. 10/031,353

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*AMENDMENTS TO THE CLAIMS*

This listing of claims replaces all prior versions, and listings, of claims in the application.

1. (Currently Amended) A high-throughput method of distinguishing at least two molecules simultaneously in a sample comprising multiple molecules, said method comprising:

(i) subjecting a sample comprising multiple molecules, at least two molecules of which are is detectably labeled, to electrophoresis in an aqueous solution, wherein said multiple molecules in said sample are not amplified prior to being subjected to electrophoresis,

(ii) imaging the electrophoretic mobility of each of the at least two detectably labeled molecules over time by detecting the position of the detectable label of each of the at least two detectably labeled molecules over time and, optionally, at the same time, dispersing the image by a transmission grating for spectroscopic analysis, and

(iii) determining the electrophoretic mobility of each of the at least two detectably labeled molecules ~~and, optionally~~, optionally determining the molecular spectrum of each of the at least two detectably labeled molecules, ~~thereby and~~ distinguishing each of the at least two molecules individually in a sample comprising multiple molecules.

2. (Cancelled)

3. (Previously Presented) The high-throughput method of claim 1, wherein said at least two molecules are nucleic acids.

4. (Previously Presented) The high-throughput method of claim 3, wherein each nucleic acid is detectably labeled with a fluorescent label.

5. (Previously Presented) The high-throughput method of claim 1, wherein said at least two molecules are proteins.

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6. (Previously Presented) The high-throughput method of claim 5, wherein each protein is detectably labeled with a fluorescent label.
7. (Previously Presented) The high-throughput method of claim 1, wherein said at least two molecules are small molecules.
8. (Previously Presented) The high-throughput method of claim 1, wherein said sample comprises a buffer.
9. (Original) The high-throughput method of claim 8, wherein said buffer is photobleached.
10. (Original) The high-throughput method of claim 8, wherein said buffer comprises a sieving matrix.
11. (Previously Presented) The high-throughput method of claim 1, wherein said at least two molecules are detectably labeled with a fluorescent label and said fluorescent label is induced to fluoresce by a laser.
12. (Previously Presented) The high-throughput method of claim 11, wherein fluorescence from said fluorescent label is focused on an imaging means.
13. (Previously Presented) The high-throughput method of claim 12, wherein said imaging means is an intensified CCD camera.
14. (Previously Presented) The high-throughput method of claim 12, wherein said laser generates extraneous light and said extraneous light is eliminated through the use of an equilateral prism and at least one optical pinhole positioned before said imaging means.
15. (Previously Presented) The high-throughput method of claim 12, wherein one or more optical filters are positioned in front of said imaging means.

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16. (Previously Presented) The high-throughput method of claim 1, wherein said electrophoretic mobility is measured by a method selected from the group consisting of the multiframe method, the streak method and the multispot method.

17. (Previously Presented) The high-throughput method of claim 1, wherein said electrophoretic mobility is imaged in less than about 5 milliseconds.

18. (Currently Amended) The high-throughput method of claim 1, wherein each of the at least two detectably labeled molecules are present in said sample at a concentration of at least about 1 copy per milliliter.

19. (Currently Amended) The high-throughput method of claim 1, wherein each of at least about 200 detectably labeled molecules is ~~are~~ imaged every 10 milliseconds.

20. (Currently Amended) The high-throughput method of claim 19, wherein each of at least about 2,500 detectably labeled molecules is ~~are~~ imaged every 25 milliseconds.

21. (Currently Amended) A system for use in the method of claim 1, said system comprising:

(i) an electrophoretic sample channel, into which is introduced a sample comprising multiple molecules, at least two molecules of which are detectably labeled with a fluorescent label, wherein said multiple molecules in said sample are not amplified prior to being introduced into the electrophoretic sample channel,

(ii) a light source comprising or consisting essentially of at least one wavelength of light that causes each of the at least two molecules in said sample comprising multiple molecules that is detectably labeled with a fluorescent label to fluoresce, wherein said light source irradiates said electrophoretic sample channel,

(iii) an imaging means, wherein said imaging means images the electrophoretic mobility of each of the at least two detectably labeled molecules in said sample over time, and, optionally,

(iv) a transmission grating, which disperses the imaging of the electrophoretic mobility of each of the at least two detectably labeled molecules in said sample.

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22. (Original) The system of claim 21, which further comprises a lens between said light source and said electrophoretic sample channel, wherein said lens focuses said light at normal incidence to said electrophoretic sample channel.

23. (Previously Presented) The system of claim 21, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

24.-56. (Cancelled)

57. (Previously Presented) The system of claim 22, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

58. (Previously Presented) The system of claim 21, wherein said imaging means is an intensified CCD camera.

59. (Previously Presented) The system of claim 58, which further comprises a microscope objective between said electrophoretic sample channel and said imaging means, wherein said microscope objective focuses the fluorescence from said fluorescent label onto said imaging means.

60. (Previously Presented) The system of claim 21, which further comprises one or more optical filters positioned in front of said imaging means.

61. (Previously Presented) The system of claim 22, which further comprises one or more optical filters positioned in front of said imaging means.

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62. (Currently Amended) The system of claim 21, wherein said imaging means images the electrophoretic mobility of each of at least two detectably labeled molecules in said sample in less than about 5 milliseconds.

63. (Currently Amended) The system of claim 21, wherein said imaging means images the electrophoretic mobility of each of at least about 200 detectably labeled molecules every 10 milliseconds.

64. (Currently Amended) The system of claim 21, wherein said imaging means images the electrophoretic mobility of each of at least about 2,500 detectably labeled molecules every 25 milliseconds.

65. (Currently Amended) A high-throughput method of distinguishing at least two molecules simultaneously in a sample comprising multiple molecules, said method comprising:

- (i) introducing a sample comprising multiple molecules in aqueous free solution, at least two molecules of which are detectably labeled, into a sample channel, wherein said multiple molecules in said sample are not amplified prior to being introduced into said sample channel, and wherein the sample is maintained in the sample channel as an aqueous solution,
- (ii) simultaneously imaging the position of each of the at least two detectably labeled molecules by detecting the position of the detectable label of each of the at least two detectably labeled molecules and dispersing the image by a transmission grating for spectroscopic analysis, and
- (iii) determining the molecular spectrum of each of the at least two detectably labeled molecules, ~~thereby~~ and distinguishing each of at least two molecules simultaneously in a sample comprising multiple molecules.

66. (Cancelled)

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67. (Previously Presented) The high-throughput method of claim 65, wherein said at least two molecules are nucleic acids.

68. (Previously Presented) The high-throughput method of claim 67, wherein each nucleic acid is detectably labeled with a fluorescent label.

69. (Previously Presented) The high-throughput method of claim 65, wherein said at least two molecules are proteins.

70. (Previously Presented) The high-throughput method of claim 69, wherein each protein is detectably labeled with a fluorescent label.

71. (Previously Presented) The high-throughput method of claim 65, wherein said at least two molecules are small molecules.

72. (Previously Presented) The high-throughput method of claim 65, wherein said sample comprises a buffer.

73. (Previously Presented) The high-throughput method of claim 72, wherein said buffer is photobleached.

74. (Previously Presented) The high-throughput method of claim 65, wherein said at least two molecules are detectably labeled with a fluorescent label and said fluorescent label is induced to fluoresce by a laser.

75. (Previously Presented) The high-throughput method of claim 74, wherein fluorescence from said fluorescent label is focused on an imaging means.

76. (Previously Presented) The high-throughput method of claim 75, wherein said imaging means is an intensified CCD camera.



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77. (Previously Presented) The high-throughput method of claim 75, wherein said laser generates extraneous light and said extraneous light is eliminated through the use of an equilateral prism and at least one optical pinhole positioned before said imaging means.

78. (Previously Presented) The high-throughput method of claim 75, wherein one or more optical filters are positioned in front of said imaging means.

79. (Previously Presented) The high-throughput method of claim 65, wherein said position is imaged in less than about 0.05 milliseconds.

80. (Previously Presented) The high-throughput method of claim 65, wherein each of the at least two detectably labeled molecules is present in said sample at a concentration of at least about 1 copy per milliliter.

81. (Currently Amended) The high-throughput method of claim 65, wherein each of at least about 200 detectably labeled molecules is ~~are~~ imaged every 0.10 milliseconds.

82. (Currently Amended) The high-throughput method of claim 81, wherein each of at least about 2,500 detectably labeled molecules is ~~are~~ imaged every 0.25 milliseconds.

83. (Currently Amended) A system for use in the method of claim 65, said system comprising:

- (i) a sample channel, into which is introduced a sample comprising multiple molecules in free solution, at least two molecules of which are detectably labeled with a fluorescent label, wherein said multiple molecules in said sample are not amplified prior to being introduced into said sample channel,
- (ii) a light source comprising or consisting essentially of at least one wavelength of light that causes each of the at least two molecules in said sample comprising multiple molecules that are detectably labeled with a fluorescent label to fluoresce, wherein said light source irradiates said sample channel,
- (iii) an imaging means, wherein said imaging means images the position of each of the at least two detectably labeled molecules in said sample, and,

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(iv) a transmission grating, which simultaneously disperses the imaging of the position of each of the at least two detectably labeled molecules in said sample.

84. (Previously Presented) The system of claim 83, which further comprises a lens between said light source and said sample channel, wherein said lens focuses said light at normal incidence to said sample channel.

85. (Previously Presented) The system of claim 84, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

86. (Previously Presented) The system of claim 83, wherein said imaging means is an intensified CCD camera.

87. (Previously Presented) The system of claim 86, which further comprises a microscope objective between said sample channel and said imaging means, wherein said microscope objective focuses the fluorescence from said fluorescent label onto said imaging means.

88. (Previously Presented) The system of claim 83, which further comprises one or more optical filters positioned in front of said imaging means.

89. (Currently Amended) The system of claim 83, wherein said imaging means images the position of each of the at least two detectably labeled molecules in said sample in less than about 0.05 milliseconds.

90. (Currently Amended) The system of claim 83, wherein said imaging means images the position of each of at least about 200 detectably labeled molecules every 0.10 milliseconds.



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91. (Currently Amended) The system of claim 83, wherein said imaging means images the position of each of at least about 2,500 detectably labeled molecules every 0.25 milliseconds.